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ON THE EFFECT OF EXTERNAL CONDITIONS ON THE DEVELOPMENT
OF FROG EGGS

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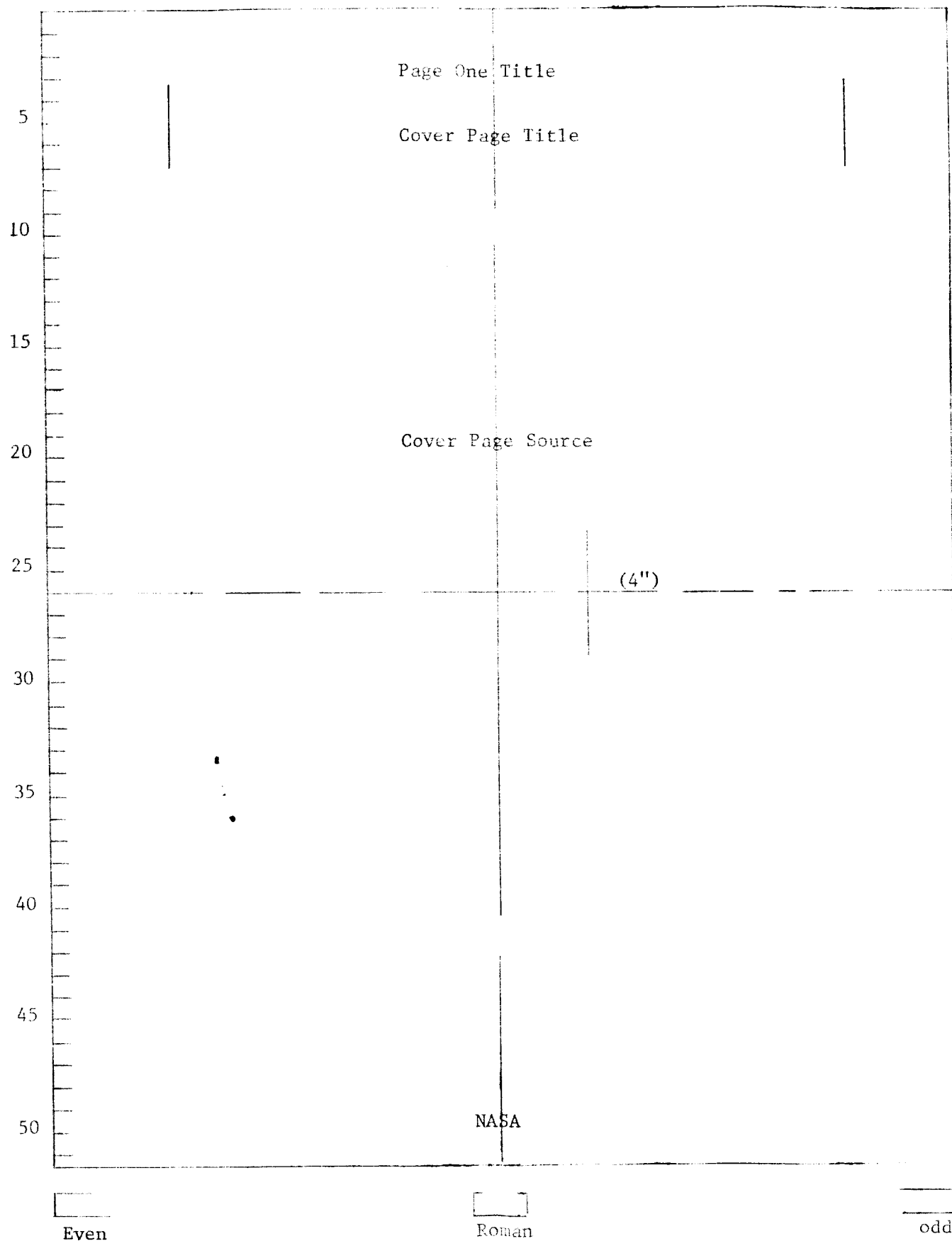
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FACILITY FORM 602



ON THE EFFECT OF EXTERNAL CONDITIONS ON THE DEVELOPMENT
OF FROG EGGS

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ABSTRACT: The effects of temperatures and saline solutions on the eggs of *Rana fusca* are investigated. The eggs develop normally but at an accelerated rate up to 25°C. Higher temperatures retard or halt the developmental process, starting in the vegetative portion. Cooling produces similar results. Saline solutions (6-10 g NaCl/100 H₂O) also attacked the vegetative portion of the eggs first. NaCl solutions produced brain damage in frogs (hemicephalia).

Since the eggs of our common brown grass frog, the *Rana fusca*, are characterized by their considerable resistance against various external effects, they are highly suitable objects for physiological experiments. Consequently, in March, following investigations on which I had reported during the previous year to the Academy, I conducted a number of experiments on this object. At that time I tried to change the external shape of these eggs by compression of the newly-fertilized eggs and to change the normal segmentation process. Based on the factual material so obtained, I tried to expose the untenability of the so-called mosaic theory developed by Roux under whose guise the preformation study of the previous century was again introduced under a more modern cloak in science.

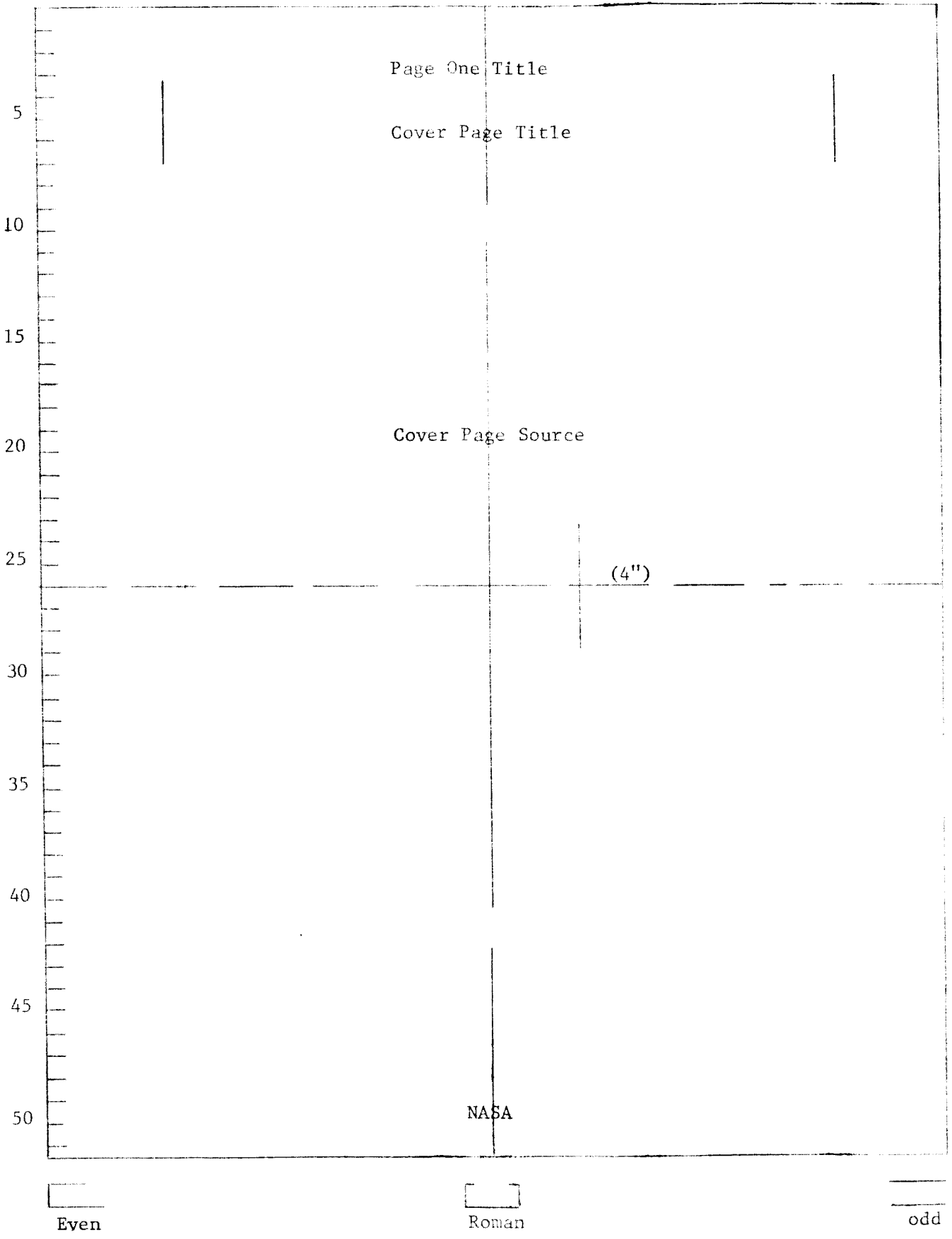
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This time I investigated, first, the effect of various temperatures, and, second, the effect of salt solutions on the development of the frog egg. The observations covered partly the living objects, partly the eggs which during the experiment were hardened daily in chromic acid and which were marked following further processing and cut into sections. Although the material has not yet been fully evaluated with regard to this latter criterion, the following can be reported regarding the major results obtained so far:

The maximum temperature at which the frog eggs develop under normal but accelerated conditions is 25°C. One batch of eggs cultivated at this temperature in the thermostat after 24 hours reached a development stage attained by other eggs cultivated at normal room temperature of 16°C only at the end of the second day. The entire gastrulation process has already been completed to the point where the remainder of the blastopore is detected only as a small white point on the evenly black surface of the yolk.

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*Numbers in the margin indicate pagination in the foreign text.



Temperatures from 25 to 30°C have a detrimental effect if applied for an extended period of time, while temperatures of 30 to 35°C act in the same manner on the egg material after a short period of time. The damage consists at first of a slowdown and, after prolonged exposure to the detrimental factor, of a complete paralyzation of the development process which leads to a gradual destruction of the objects.

The damage does not affect the entire egg evenly, but at first and mainly that half which is considered the vegetative portion. This is noted as we progress in our research process if the investigation material is placed in a bowl of water and this bowl into a thermostat soon after fertilization at a temperature of 28 to 30°C. While the animal, pigmented half of the yolk divides more or less regularly into pieces according to partial planes, the vegetative half is not divided and this division may, even though it has already begun, be made partially ineffective in that the yolk fragments begin to melt at the separation surfaces.

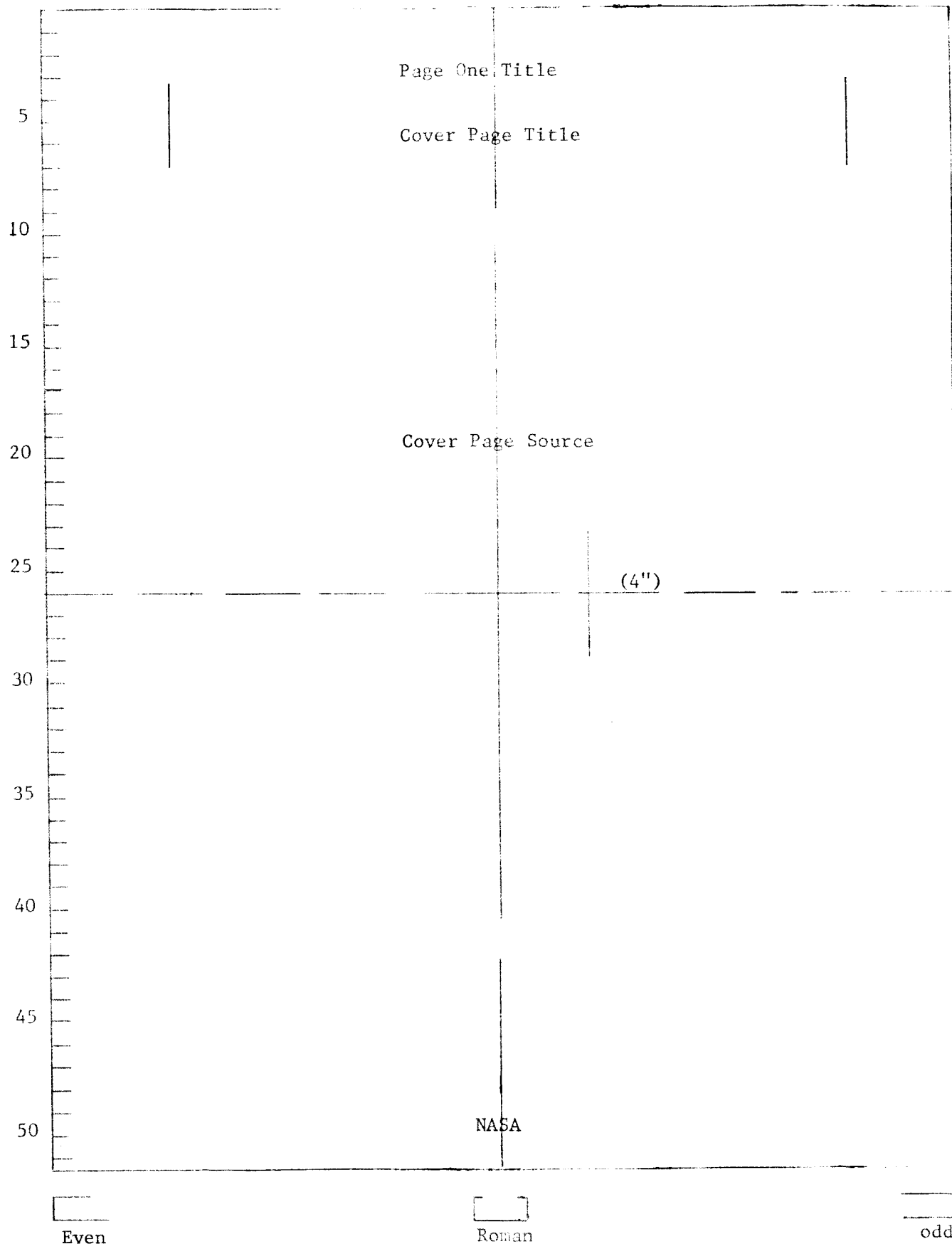
If eggs which as a result of excessive temperature have undergone partial damage are removed from the thermostat (after two to three hours at a temperature of 30°C, after three to eight hours at temperatures from 26 to 28°C) and if the bowl is now allowed to stand at room temperature, part of the material can still recover from the damage suffered; the development process continues normally, but it is mostly retarded in comparison with eggs which have developed from the beginning at room temperature.

Furthermore, among the eggs investigated, depending on the degree of intervention, there are more or less numerous eggs with permanent damage. It consists mainly of the following: In the vegetative half of the yolk, a larger or smaller area has become incapable of development and dies off while the remainder of the egg continues developing and forms cotyledons, a nervous system, and notochord. Often this leads to various partial formations similar to those obtained if the experimenter punctures the egg during the segmentation process, as first mentioned by Roux with a heated needle and kills off one of the two or four first segmentation cells either in toto or in part. Here as there, the dead material reaches the ventral surface and the posterior end of the embryo.

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Finally, eggs are also encountered which have been damaged so much that they are unable to develop beyond the germinal stage. The germinal vesicle then consists of a vegetative half which has remained undivided and a layer of small pigmented cells which cover it. The whole can then be compared to the corresponding development stage of a meroblastic egg. Generally, this is later followed by decomposition.

The fact that frog eggs are at first partially damaged at increased temperature and that they may perhaps die off, can evidently be attributed to the unequal organization of the animal and vegetative halves of the yolk. The animal half of the yolk is richer in protoplasm and is more firmly controlled by the cell nucleus. Under the normal interaction between the protoplasm and the nucleus, damage experienced by a cell can be repaired again, as has been



determined by numerous experiments. In this regard, the vegetative half of the yolk is better off. Here, the protoplasm is not only distributed more sparsely between the yolk platelets but in the undivided egg it is also farther removed from the effect of that cell nucleus which lies in the animal half; later on, following the first segmentation stadia, the fragments are many times larger than the cells formed from the animal half of the egg.

From these organization conditions of the frog egg, the rather odd and constant phenomenon can be explained satisfactorily that it is always the vegetative half of the egg which decomposes under heat catalepsy and that here too, a smaller or larger area always dies off at first.

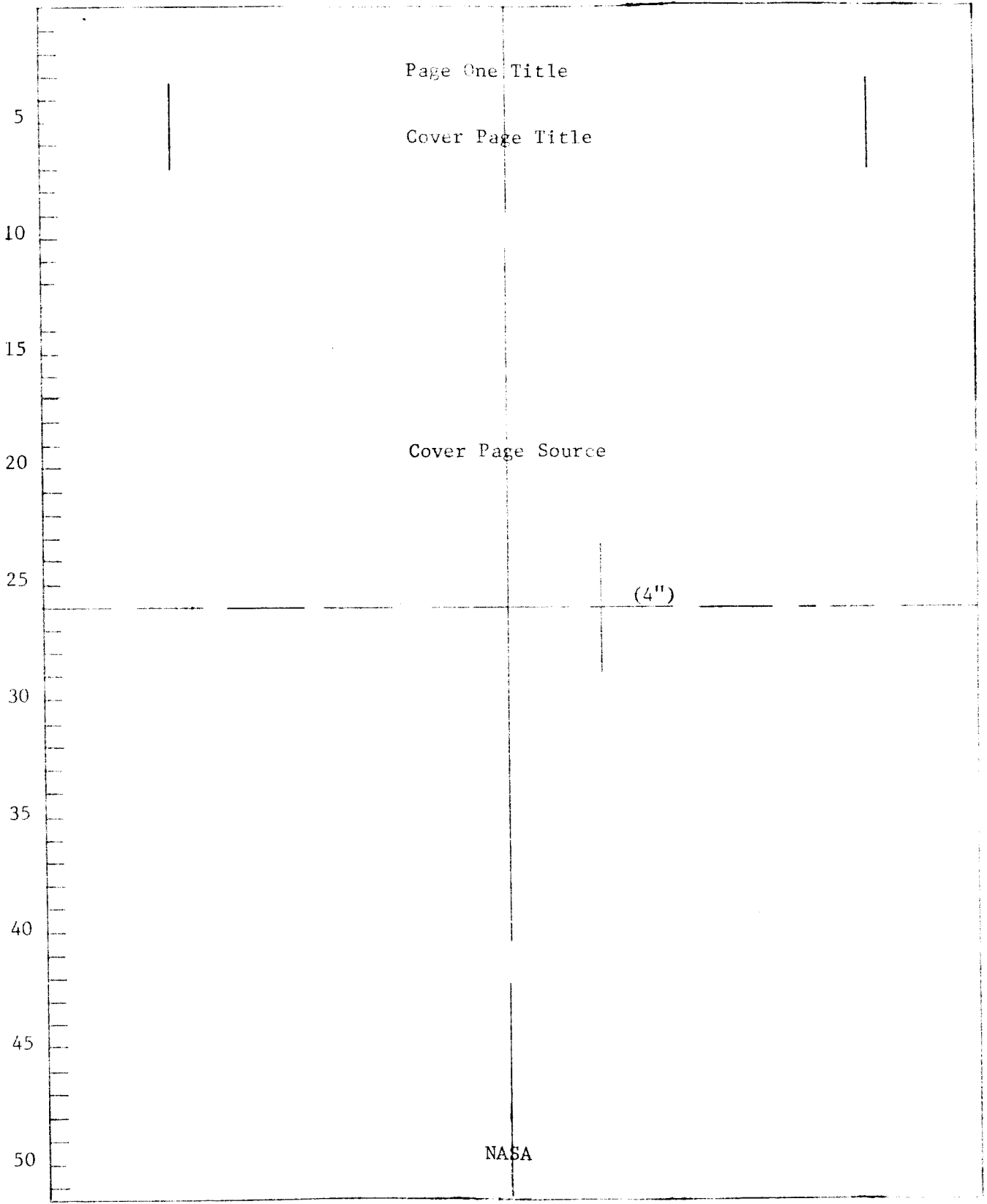
Similar results as those stemming from increased temperatures can be obtained by a pronounced cooling of the eggs. In one experiment, a batch was placed in a refrigerator shortly after they had been fertilized, and kept at a water temperature of 0° for 24 hours. No division of the egg was noted. The development process was completely paralyzed in every direction, but it started again when on the following day the eggs were removed from the refrigerator and further cultivated at room temperature. Only now, normal division by two and four, etc., occurred.

However, damage was inflicted on the eggs due to the long cooling at 0° and the freezing and paralyzation of the development process. In many respects, they are equal in their behavior to those eggs which were heated for some time at 25°C. In one batch, the development indeed proceeded normally, but considerably slower than in eggs which were kept at room temperature from the beginning. In another batch, a more or less large area of the vegetative half of the yolk was permanently damaged so that it could not be divided into cells and it had to be separated from the healthy developing batch slowly since it was useless with respect to the development process.

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Odd and in many ways interesting results stemmed from experiments made with kitchen salt solutions of different concentrations. I soon discovered a fixed starting point in a study performed by the U. S. scientist Morgan in conjunction with the Japanese scientist Ume Tsuda and which was just published under the heading "The Orientation of the Frog's Egg" in the last issue of the Quarterly Journal of Microscopical Science. These researchers tested, among other things, the effect of various chemicals on the development of frog eggs and found that kitchen salt leads to interferences in the formation of the blastopore. Instead of closing normally, it remains largely open even during later development phases. The proper solution is obtained by adding three grams of kitchen salt to 500cc of water.

In my experiments, I used the same concentration, but also solutions of 4:500 and 5:500. Only in the weaker solutions the eggs could be cultivated for several days, while in the 5:500 concentration, they indeed began to segment normally, but they died off soon thereafter. Here, too, the damaging effect of the stronger salt solution affected at first the behavior of the vegetative half of the egg just as in the tests with higher or lower temperatures.



Due to the effect of a 3:500 kitchen salt solution, mainly two very peculiar disturbances in the development process were produced. One disturbance concerns the formation of the blastopore and the other the development of the brain.

Let us first discuss the first disturbance. The normal blastopore formation process, as we well know, consists of the fact that a folding over is produced at the downward-facing surface of the germinal vesicle at that edge where the pigmented part of the surface becomes the unpigmented part or where it converts into the yolk. This foldover increases in size slowly starting at that point where it first started and which becomes the head of the embryo. It develops backwards in that it covers the entire yolk field; at the same time, the first edges of the foldover or of the blastopore grow from the left and from the right toward each other and merge in the later median plane of the embryo in the blastopore seam from the front toward the rear. Thus, the yolk field is slowly overgrown and absorbed in the archenteron of the embryo so that now the entire egg surface is pigmented black.

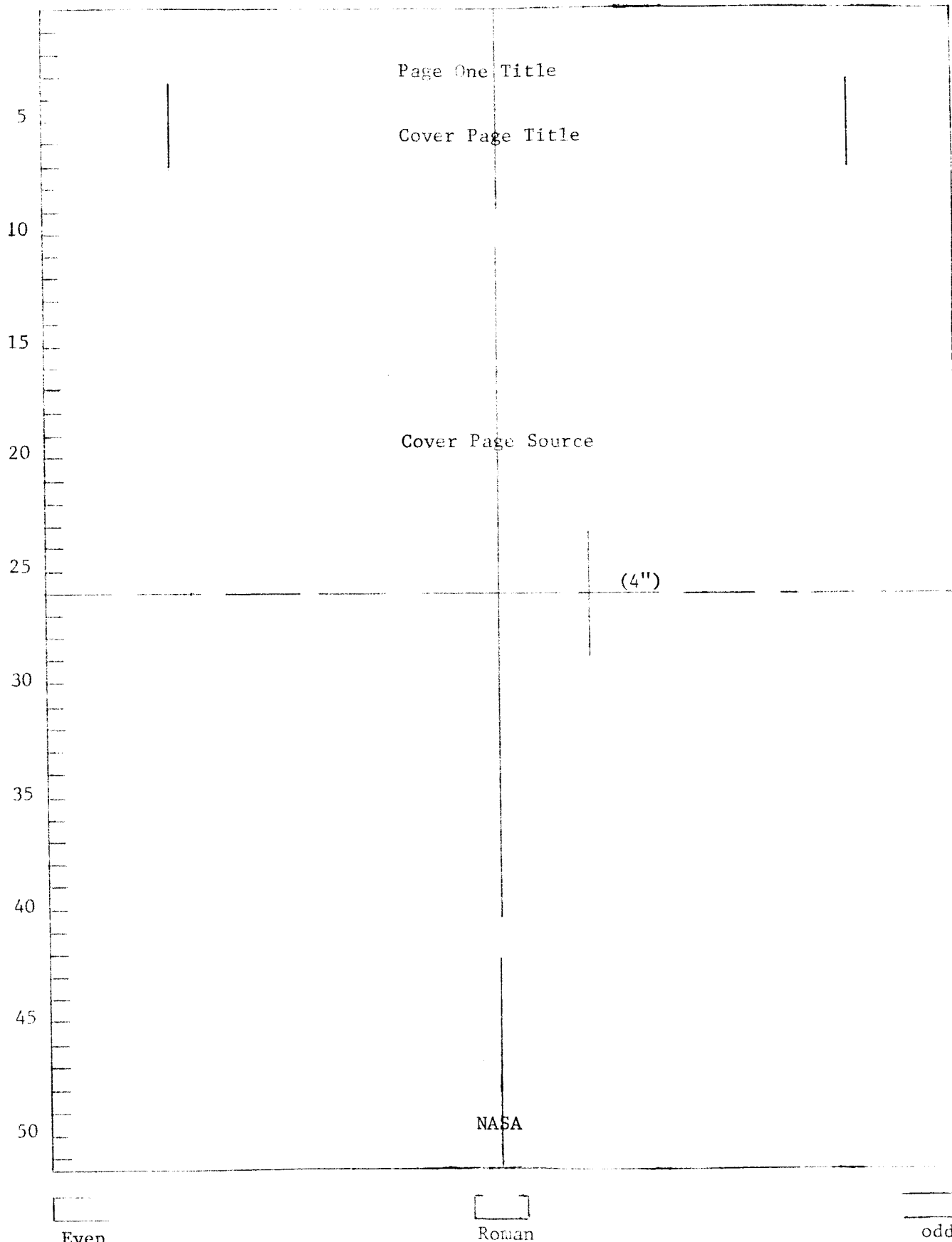
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In our deformations, however, the foldover which yields the archenteron remained limited to a smaller spot at the edge of the yolk field; this is also where the enlargement of the embryonal body begins. The yolk field remains free over the entire extent at the egg surface; in the embryo formation, it is not overgrown by the blastopore edges and it can be seen in its original size even in the five-day-old embryos which have already developed the head, the layout of the sensory organs, the notochord, the neural ectoderm, and many embryonic segments; it then occupies the portion opposite the head, namely at the rear.

In the free position of the yolk field, the eggs then are equal to the deformations which was the object of my paper entitled "Blastopore and *Spina bifida*" resulting from a more detailed investigation several years ago. Morgan also compared them to each other. Upon a rigorous examination, the agreement turns out to be only quite superficial; in reality the formation steps are fundamentally unequal.

In embryos with *Spina bifida*, the entire circumference of the yolk field was transformed in the blastopore edge and from it the formation of the middle cotyledon begins everywhere; half a medullar plate, the notochord, and a number of embryonic segments differentiate here. Since the yolk field in this case was not overgrown by blastopore edges, the entire back of the embryo consists of two halves separated along their entire length by a wide cleavage through which we reach the intestinal space filled with yolk mass. By a cicatrization of the two embryo halves, the highest degree of *Spina bifida* can be converted more or less into a norm.

In the deformations produced by the kitchen salt solution (3:500), however, the blastopore edge, to the degree that from it the formation of the embryonal axial organs originates (neural ectoderm, notochord, embryonal segments) fails to surround the entire periphery of the yolk field, at least up to the final stage investigated by this author, but it remains limited to a



smaller area. In general, the process is similar to that of meroblastic eggs of selachians, reptiles, and birds. Just as we are able to divide the edge of the embryonic disc into a blastopore edge and into a transformation edge in these objects, a similar classification can be made at the periphery of the yolk field of the frog eggs developing abnormally as a result of the kitchen salt. One batch will be used for embryo formation (blastopore edge); in the other which is comparable to the transformation edge of the meroblastic eggs, no formation of notochord, medullar membranes, or embryonic segments occurs as in the other category of deformations. In the embryos developed in kitchen salt solution, the dorsal organs therefore never exhibit *Spina bifida*. The notochord is simple and the medullar tube is closed. Just as the caudal flap rises as in the selachians, here too the posterior end of the lengthwise-growing embryo rises above the surface of the yolk field in the form of a hunchback which grows rapidly in the longitudinal direction and protrudes considerably beyond the yolk field which is still free, whereby the deformation now assumes an even more peculiar and strange aspect.

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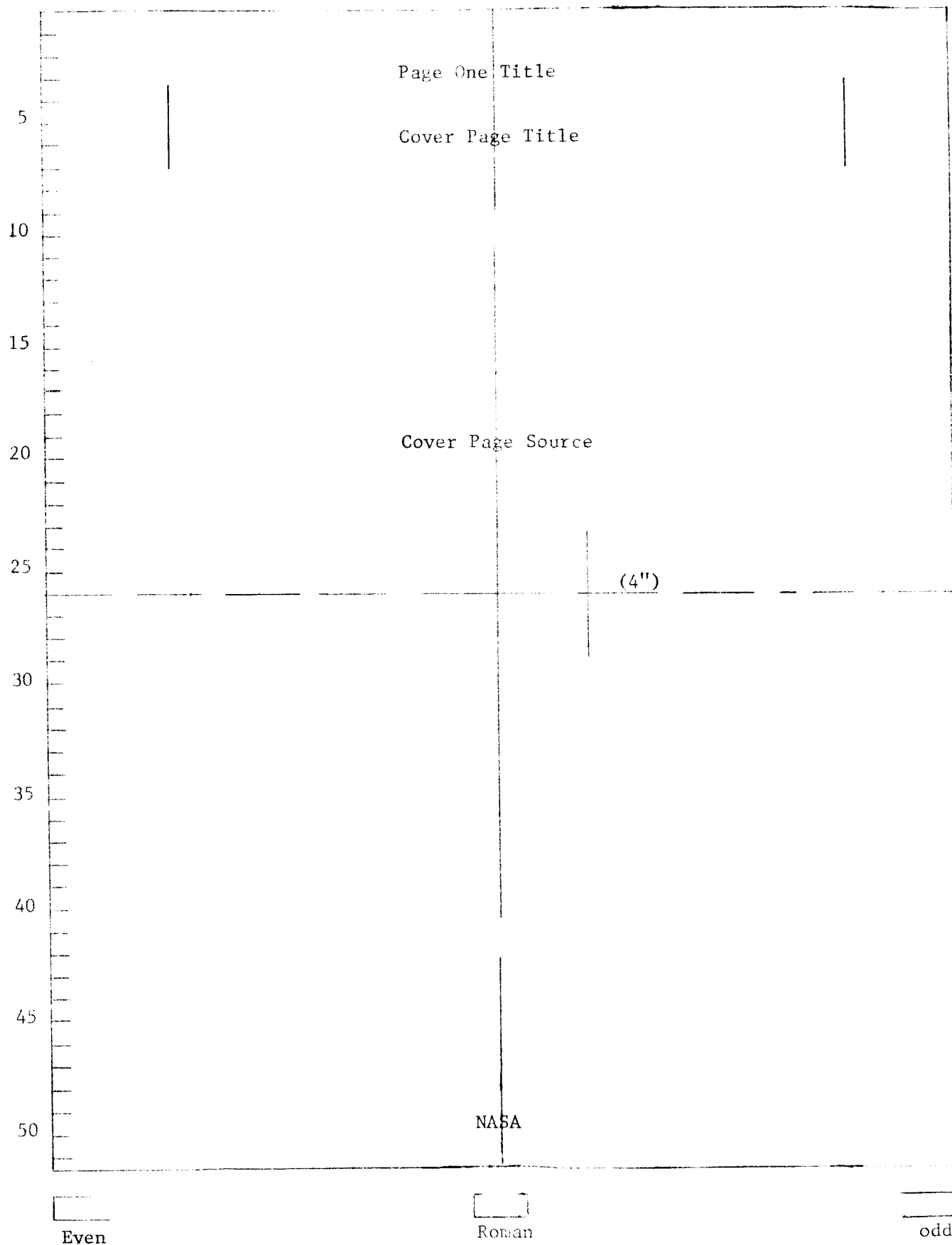
Upon more detailed examination it becomes evident that, depending on whether the egg developed normally or in one way or another disturbed, the cellular material produced by the segmentation process becomes useful in a highly differentiated way for the buildup of the embryonal body. This is new evidence for the untenability of Roux's mosaic theory or of the determinant philosophy propounded by Weismann.

The second disturbance already mentioned by me is produced by the effect of the kitchen salt on the development of the brain. All embryos, in addition to the free yolk field, exhibit the interesting abnormality which in human teratology is called hemicephalia and anencephalia. In the vicinity of the middle brain, the cerebellum, and the medulla oblongata, the medullar membrane has not formed a folded joint and a closed tube as should have happened under normal circumstances, and as did happen in the frontal part of the brain and in the layout of the spinal cord but rather, it remained flat and is hemmed in only by a puffed-up area which protrudes beyond the surface and at which the neural tissue turns into the cornual folium.

In humans and in mammals, deformations with hemicephalia has been observed only in a highly advanced phase. Therefore, hypotheses had to be depended on when answering the question how hemicephalia is produced during the development process. As seen from the literature, these hypotheses differed markedly from each other.

Therefore, the artificial production of hemicephalia in frog embryos appears to be of great interest since here the production of the deformation could be accurately followed from its inception. As can be clearly seen, a deformation of the impediment type is clearly involved. In the vicinity of the later cerebellum and medulla oblongata, the brain layout remains intact as a membrane as can be determined from causes which must be still further investigated, hemmed in by puffed-up edges which form the transition to the epidermis. Since the closed spinal cord is never formed, the connective tissue in which the bony cranial roof will be located later on cannot

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develop in this part of the head. Consequently, the cranial roof will then be absent while the ossified basis cranii is fully formed and covered by the brain masses which developed extraordinarily well from the medullar membrane under these extraordinary conditions.

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